CERHR DRAFT EVALUATION OF
DI-ISONONYL PHTHALATE

May 24, 2000

Di-Isononyl Phthalate—Contents

1.0 EXPOSURE

1.1 Chemistry

DINP is a complex substance assigned two different CAS numbers. CAS number 68515-48-0 (designated DINP-1 in this document) is manufactured from octene that is converted to alcohol moities consisting mainly of 3,4-, 4,6-, 3,6-, 3,5, 4,5-, and 5,6-dimethyl-heptanol-1. The CAS number 28553-12-0 (DINP-2) is produced from n-butene that is converted primarily to methyloctanols and dimethylheptanols. The 28553-12-0 CAS number also represents DINP-3 which is produced from n-butene and isobutene that are converted to alcohols, with 60% consisting of methylethyl hexanols. The CMA (*1*) has stated that although DINP is a complex substance, it is not variable due to the stability of the alcohol manufacturing process. The two types of DINP are considered commercially interchangeable.

DINP is an oily, viscous liquid at standard temperature and pressure.

Table 1: Physico-chemical Properties of DINP

Property	Value
Chemical Formula	$C_{26}H_{42}O_4$
Molecular Weight	419
Melting Point	-48 ^oC
Boiling Point	370 ^oC
Specific Gravity	0.97
Solubility in Water	Insoluble (<0.001 mg/L)
Log K_{ow}	~9

(*2*)

1.2 Exposure

Humans may be exposed to DINP by the oral, dermal, and inhalation routes of exposure. Occupational exposure occurs primarily through inhalation and dermal contact, while consumer exposure occurs primarily by oral and dermal routes. Exposure of children to DINP through children's products is a concern of the public.

Occupational exposure. DINP, like other phthalate esters, is manufactured within a closed system under negative pressure. However, some exposures may occur during the loading and unloading of railroad cars and trucks. Slightly higher exposures may occur during the production of PVC products because of elevated temperatures and more open processes. CMA (*1*) cites six studies that indicate exposures are below 1 mg/m^3 during production of phthalates and below 2 mg/m^3 during production of PVC. As discussed in Section 2.3, dermal exposure is not expected to result in significant absorption into the body.

Consumer exposure. The range of products that contain DINP is quite broad. The use, categories, and amounts used of DINP in 1998 are given in Table 2.

Table 2: Calculated 1998 US Consumption of DINP
(thousands of metric tons)

End Use	Subtotal	Total
Film and Sheet		13
Stationary and Wood Veneer	6	
Pool Liners	1	
Other	6	
Flooring		48
Tiles	23	
Sheets	25	
Artificial Leather		3
Coated Fabrics		21
Tarps	16	
Conveyor Belts	1	
Other	4	
Dip Coating/Slush Molded		30
Gloves	15	
Toys	6	
Traffic Cones	<1	
Other	~9	
Tubings and Profiles		7
Profiles	5	
Garden Hoses	2	
Wire and Cables		32
Shoes/Shoe Soles		9
Under-Body Coating		7
Sealants (carpet backing)		8
GRAND TOTAL		178

(*1*)

DINP is a general purpose plasticizer with a broad range of applications used in flexible PVC. It is widely used in the toy, construction, and general consumer product markets. It has limited use in food packaging and is not used in medical applications.

General exposure to DINP is probably very similar to exposure to DEHP, but few monitoring data were located. It is assumed that environmentally-contaminated food represents the major human exposure, followed by indoor air, ambient air, and water.

DINP's solubility in water is extremely low; levels are often below the analytical detection limit. Vapor pressure is also extremely low, so measured concentrations in air are not available. Modeling based on physico-chemical properties of DINP can be compared to similar models for DEHP.

Food. In 1996, dinonyl phthalate (isomer not specified) was identified but not quantified in 4 of 12 infant formulas from the UK (*3*). In a follow-up survey conducted by MAFF (*4*), DINP was not specifically targeted but there was no evidence of its presence in 39 samples of infant formula from the UK.

Toys. PVC plastics are often used in children's products. Different phthalates are constituents of PVC; DINP is currently the predominant plasticizer (*5*). Other phthalates, including DEHP, have been or are also

used (*6, 7*). However, US toy manufacturers began voluntary removal of DEHP from pacifiers and nipples in 1986 (*8*). Few studies pertaining to plasticizers in children's products were found in the peer reviewed literature. Additional information is available from industry groups and several government agencies. The Expert Panel did not perform a comprehensive review of available data but believes the information it reviewed reflects the general state of knowledge.

As reported by CPSC (*5*), Chen measured DINP in 31 of 35 toys and found a concentration range of 15.1–54.4 % dry weight. Health Canada (*9*) analyzed 41 children's products made in the US, China, and Thailand for the presence of DINP and DEHP. DINP was detectable in 27 of the 41 products in concentrations that ranged from 3.9 to 44% dry weight. DEHP was detected in 23 of the products at lower concentrations of 0.002–0.19% dry weight. DEHP was found in 1 of 5 US-made, 22 of 35 Chinese, and 1 of 2 Thai products. Criteria for the selection of products were not discussed in any of these surveys. No information on market share, length of availability on the market, or estimates of the numbers of products in circulation was noted in any study. Only Health Canada listed product number, country of origin, manufacturer/distributor, and brand. All studies listed a product description. Marin (*7*) analyzed 15 samples of materials used in toys in Spain. The authors noted that the PVC contained a mixture of plasticizers including DINP, DEHP, and DIDP, but reported only the DEHP content. A range of <0.1–34% DEHP dry weight was reported and 6 of 15 samples contained >10% DEHP dry weight. Rastogi (*6*) found DINP and DIDP in 4 of 4 teethers (31.6–40.2% w/w), and 2 of 3 dolls (19.6 and 26.5% w/w). He also found DEHP in 3 of 4 teethers (0.01–0.07 % dry weight) and 2 of 3 dolls (0.12 and 22.4 % dry weight). DBP was present at 0.01% in one doll, DEP was present at 0.13% in one teether and BBP was present in one doll at 0.02 % dry weight. Lay (*10*) found 4 commercially available pacifiers contained 31.4–41.6% dry weight DEHP. Since this paper was published in 1987, it is likely that the pacifiers studied are no longer available in the US.

The estimation of actual exposure of children to phthalates contained in children's products has been studied. *In vitro* studies using various agitation and impaction approaches yield a wide range of extraction of DINP from toys. CPSC used stainless steel pistons, 11 cm^2 of each product, and simulant saliva to obtain extraction rates for their 31 DINP positive children's products. Migration was log normally distributed with a mean rate of 8.2 〉 9.83 µg/11 cm^2/hour and a range of 1–48 µg/11 cm^2/hour. Both CPSC (*5*) and Health Canada (*9*) failed to find any correlation between release rate of DINP under experimental conditions and total DINP content.

Steiner et al. (*11*) measured migration of DEHP into a saliva simulant under static and dynamic conditions (simple shaking with glass balls and glass plates, and simulated chewing with glass dentures) using a standardized PVC film. They also performed three 3-hour and two 6-hour sucking tests using the same adult volunteers, collecting all saliva. The range of extraction varied by a factor of 40 among the various experimental scenarios. Adult sucking was comparable to static methods which were the lowest migrations at 64 ± 14 µg DEHP/dm^2 film and 41 ± 9 µg DEHP/g film, respectively. The highest dynamic extraction resulted in 1,006 ± 484 µg DEHP/g film.

The Dutch Consensus Group reported a small study by Meuling and Rijk (*12*) using 20 adult volunteers. A control specimen without DINP and three specimens with DINP were used; specimen 1 contained 38% DINP. Specimens 2 and 3 came from different parts of the same commercially-available teething ring, representing different shapes for mouthing and contained 43% DINP each. All three were 10 cm^2 total area. All 20 volunteers were instructed to suck and bite on the control specimen for 10–15 minutes, all saliva was collected, then volunteers rested 5 minutes and then they performed 4 separate sessions on the same test piece of specimen 1 resting 5 minutes between each session. This procedure was repeated with half (n=10) of the volunteers on specimen 2 and the other half (n=10) on specimen 3. DINP extraction from specimen 1 was 1.38 (0.3–8.3) µg/min, from specimen 2 2.44 (0.9–8.9) µg/min, and from specimen 3 1.63 (0.9–5.7) µg/min. The mean across all groups was 1.8 µg/10cm^2/min (or 120 µg/11cm^2/hour). There was no

correlation between extraction and pH or protein content of the saliva. Release rates over the various 15-minute intervals seemed consistent. The increase in extraction of Specimen 2 was thought to be due to the finger-like shape resulting in different mouthing behaviors from those employed on the disk-like shape of Specimens 1 and 3.

CPSC (*5*) reported a similar protocol using 10 adult volunteers and 5 toys, and found a mean migration rate of 241.3 µg/11cm^2/hour. This rate was 39.5 times higher than the average rate obtained by impaction with disks cut from the same 5 toys, but was similar to the ranges in the Dutch simulation study.

CPSC (*5*), the Dutch Consensus Group (*12*), and Health Canada (*9*) have attempted to calculate daily intake based upon the leaching rates described above. The Dutch Group used Monte Carlo simulation and estimates of mouthing time and the leaching rates from the *in vivo* study of 20 adults. Mouthing time was derived from parent observations and logging of mouthing time of 42 children aged 3–35 months. Mouthing time was calculated for the time children were awake, but not eating, during ten 15 minute observation periods over 2 days. Logs were kept of objects mouthed; the objects were divided into those intended for mouthing and those not intended for mouthing. The Dutch calculations used total mouthing time.

Table 3: Total Mouthing Time

Age (months)	Sample Size	Mean in Minutes (SD)	Min (Minutes)	Max (Minutes)
3–6	5	36.9 ±19.1	14.5	67.0
6–12	14	44.0 ± 44.7	2.4	171.5
12–18	12	16.4 ± 18.2	0	53.2
18–36	11	9.3 ± 9.8	0	30.9

Because the greatest exposure levels were determined for children within the ages of 3-12 months, the results for that age group are summarized in Table 4.

Table 4: Toy Exposure Estimates for Children Aged 3-12 Months.

	Estimated Intake Level (µg/kg bw/day)		
Agency	Mean	95th Percentile	Maximum
RIVM*	6.53—14.4	20.7—39.7	70.7—204
CPSC	5.7	94.3	-
Health Canada**	44	-	320

* Exposure range for 3–6 month old and 6–12 month old children; range includes results from 3 specimens tested.
** Calculated with mouthing times for teethers and other objects intended for mouthing.

Exposure of children to DINP from PVC toys was also estimated by Fiala et al. (*13*) in Austria. DINP levels were measured in the saliva of 10 adult volunteers who first sucked on and then sucked and chewed on 10-15 cm^2 pieces of teether (containing about 36% DINP) for one hour. In the experiment where the volunteers only sucked on the sample, the migration rates of DINP ranged from 297-1452 µg/dm^2/hour with a mean migration rate of 832 ±397 µg/dm^2/hour. Using assumptions of an 8 kg body weight, 3 hour exposure time (*12*), and 10 cm^2 mouthing area, mean and maximum exposure levels of 31.25 µg/kg bw/day and 54.4 µg/kg bw/day, respectively, were estimated. For the experiment where the adults chewed on the sample, migration rates of DINP in 9 adults ranged from 768-2152 µg/dm^2/hour. Using the same assumptions from the first experiment, a maximum exposure level of 84.5 µg/kg bw/day was estimated.

The approach taken by Health Canada used published data and 10,000 Monte Carlo simulations. CPSC used the same mouthing time data, but limited its calculations to the mouthing time of objects not intended for mouthing. They performed a log transformation of the time because of the extreme skewness in the sample and calculated a geometric mean mouthing time of 12.03 minutes (95% CI 6.2–23.3). Exposure estimates were made using a log linear model, the mean leaching rate from mechanical extraction from 31 consumer products and a 39.5 factor from the mouthing study. The differences in the analyses resulted in quite different exposure estimates, which explains the different conclusions and recommendations of the agencies.

The differences also highlight the uncertainties inherent in these calculations. Because extraction of DINP does not correlate with DINP content, because extraction is highly variable across both laboratory procedures and human subjects, and because the number and distribution of children's products containing DINP is unknown, the amounts of DINP presented to a child cannot be well characterized. Furthermore, the estimates of mouthing behavior in the youngest and potentially highest risk group, 3–12 months, are based upon only 19 children. No discussion of developmental age, physical condition, ethnicity, or other socio-demographic indicators is included in the small parental observation study. These numbers are preliminary estimates at best. Standardization of laboratory techniques with correlation with *in vivo* simulations, better data on product distribution and use, and independent studies of mouthing behavior in babies and young children are needed.

Exposure Estimate: Based on the physiochemical characteristics of DINP and limited monitoring data, the Expert Panel believes it reasonable to assume that exposure to DINP in the general adult population is lower than exposure to DEHP, which is estimated at 3–30 µg/kg bw/day (*14*).

See Section 5.1.1 for human exposure summary.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

There were no human data identified.

2.1.2 Experimental Animal Data

BIBRA (*15*) (Table WEB-1) conducted a 21-day dietary study in F344 rats where groups of 5 males and 5 females were fed concentrations of 0, 0.6, 1.2, or 2.5% DINP (M: 639, 1,192, or 2,195 mg/kg bw/day; F: 607, 1,193, or 2,289 mg/kg bw/day). The test material most likely consisted of a mixture of DINP represented by CAS numbers 68515-48-0 and 28553-12-0 (DINP-1 and DINP-2). A positive control group of 5 rats per sex was exposed to 1.2% DEHP (M: 1,084 mg/kg bw/day; F: 1063 mg/kg bw/day). Body weight and food intake were measured twice weekly. On day 21, rats were killed and necropsied. Liver, kidney, and testes were preserved in formalin and examined histologically. Peroxisomal proliferation was assessed by measuring activities of peroxisomal proliferation enzymes, and by examining liver tissue by electron microscopy.

Table WEB-1 (download all WEB-tables separately)

A significant decrease in weight gain was observed in the mid- and high-dose groups. Food intake was significantly reduced in males. Organ to body weight ratios that were significantly increased in all treatment groups included liver (M: 136, 173, and 232%, F: 131, 175, and 237% of control values) and kidney (M: 115, 122, and 124%, F: 107, 108, and 114% of control values). Histopathological changes were not observed in kidneys; changes in liver were limited to reduced cytoplasmic basophilia in the mid- and high-dose group and increased cytoplasmic eosinophilia in the high-dose group. Palmitoyl-CoA (PCoA) oxidase activity was significantly increased in the mid- and high-dose groups (M: 452 and 1,035%; F: 376 and 1,104% increase respectively compared to controls) and an increase in peroxisome numbers was observed by electron microscopy in livers from the high-dose group. The activity of 11-hydroxylase and 12-hydroxylase was significantly increased in males of all dose groups and in females of the high-dose group. Significant changes observed in all treatment groups included increased total liver proteins and reductions in serum levels of cholesterol. Serum triglyceride levels were significantly reduced in all treated males but increased in mid- and high-dose females. The testes to body weight ratio was significantly increased in the high-dose males (135% of control value). Testicular lesions were not observed with the exception of severe unilateral atrophy in one male of the mid-dose group. Treatment with 1,063–1,084 mg DEHP/kg bw/day resulted in similar effects including decreased weight gain, increased liver and kidney to body weight ratio, increased liver enzyme activities, and reduced serum levels of cholesterol and triglycerides. Moderate testicular atrophy was noted in one male. Peroxisomal proliferation is of particular interest and an increase in peroxisome numbers was observed after treatment with DEHP. PCoA activity was significantly increased to 683 and 540% of control values for males and females, respectively. The increase in peroxisomal enzyme activity in rats treated with 1,063–1,084 mg/kg bw/day DEHP was greater than that obtained by treatment with 1,192–1193 mg/kg bw/day (452 and 376% of control values in males and females, respectively).

This study provides evidence that the liver is a target organ of DINP. A pattern similar to effects noted with DEHP is seen: increased liver weight and induction of hepatic peroxisome proliferation. The testes do not appear to be a target organ at these dose levels. The study provided a LOAEL of 0.6% (607[F] and 639[M] mg/kg bw/day) and no NOAEL was identified.

In a 2-year dietary study, (*16*) (Table WEB-2) systemic effects resulting from DINP-1 exposure in adult Fischer 344 rats were evaluated. Groups of 110 rats per sex were fed diets containing 0, 0.03, 0.3, and 0.6% DINP-1 (males: 0, 15, 152, and 307 mg/kg bw/day; females: 0, 18, 184, and 375 mg/kg bw/day). Body weight and food intake were measured weekly. Ten rats/sex/group were killed and necropsied at 6, 12, and 18 months; the remaining rats were killed and necropsied at the end of the 2-year study. Evaluation of hematology, urine, and blood chemistry effects was performed at 6, 12, 18, and 24 months. Histopathological evaluations were conducted on the liver and the kidney from all dose groups and in the remaining organs of the control and high-dose groups. Evidence of peroxisome proliferation was determined by microscopic examination of livers of 2 rats/sex/group at 24 months.

Table WEB-2 (download all WEB-tables separately)

Significant reductions in body weight gain were observed in males from 18–24 months in the 152 mg/kg bw/day group and from 12–24 months in the 307 mg/kg bw/day group. Food intake levels were not reported. Survival was significantly decreased in females of the 184 and 375 mg/kg bw/day groups. Liver and kidney to body weight ratios were significantly increased throughout the study in both sexes in the mid- and high-dose groups (152–375 mg/kg bw/day). Spleen to body weight ratios were significantly increased in males and females of the high-dose group (307–375 mg/kg bw/day) at 24 months. A small but significant increase in adrenal to body weight ratio was reported for females in the 375 mg/kg bw/day group at 6–12

months, and in both sexes in the high-dose group (307–375 mg/kg bw/day) at 24 months. Adrenal weights were not listed in tables. Dose-related changes in liver included hepatocyte enlargement in high-dose males and females throughout treatment. At 24 months, dose-related liver effects included regenerative nodules and focal necrosis in males and females, and spongiosis hepatis in males. In males of the mid- and high-dose groups, consistent increases in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities were observed. However, for SGOT, statistical significance was obtained only at 6 and 12 months in the mid-dose group and at 6–18 months in the high-dose group. Increases in SGPT activity were statistically significant at 24 months in the mid-dose group and at 6 and 18 months in the high-dose group. An increased incidence of mononuclear cell leukemia (MNCL) was observed in both sexes of the mid- and high-dose groups. Peroxisome proliferation did not occur and there was no evidence of treatment-related lesions in testes or female reproductive organs. The only significant dose-related changes in hematology were a reduction in red blood cell count and hemoglobin and hematocrit values in males of the 307 mg/kg bw/day group at 24 months. Urinalysis results were not listed in tables, but authors reported increased urine volumes in high-dose males at all time points and transient increases in potassium and glucose. A NOAEL of 17 mg/kg bw/day was selected by the authors.

A second 2-year dietary feeding study in F344 rats was reported by Moore et al. (*17*) (Table WEB-3). Groups of 70–85 F344 rats/sex/group were fed concentrations of 0, 500, 1,500, 6,000, and 12,000 ppm DINP-1 (males: 0, 29.2, 88.3, 359, or 733 mg/kg bw/day; females: 0, 36.4, 109, 442, or 885 mg/kg bw/day). Body weight and food intake were measured weekly through weeks 16–17 and monthly thereafter. Standard, hematological, clinical chemistry, and urinalysis parameters were measured every 26 weeks. Peroxisome proliferation was measured in 5 rats/sex in control and high-dose groups at weeks 1, 2, and 13, and in 3–5 rats/sex in the control and 2 highest dose groups at week 104. Five rats/sex/group were sacrificed and necropsied at weeks 1, 2, and 13. Fifteen rats/sex/group were killed and necropsied at week 79. The remaining rats were sacrificed and necropsied at week 104. Another group of 55 rats/sex was exposed through diet to 12,000 ppm (males 733 mg/kg bw/day; females 885 mg/kg bw/day) DINP for 78 weeks and sacrificed at week 104 in order to determine if recovery occurs after exposure to DINP has ended. Histopathological evaluations were conducted on major organs from rats in all dose groups.

Table WEB-3 (download all WEB-tables separately)

Clinical signs of toxicity were observed in rats exposed to 359 mg/kg bw/day and greater, and included hunched posture, decreased activity, pale thin bodies, and fewer feces. Rats exposed to 733–885 mg/kg bw/day experienced a statistically significant reduction in weight gain accompanied by a decrease in food intake. Survival was significantly reduced in the high-dose males with only 54% surviving to the end of the study. The body weight effect was shown to be partially reversible because male weight gain in the recovery group was not reduced at week 104; reduced weight gain in females was less pronounced. Survival was not significantly affected in the recovery group. The authors reported that the dose-related depression of body weight gain in the two highest doses was associated with clinical chemistry findings or histomorphologic effects in liver and kidney. A significant increase in the incidence of anemia, as observed by decreases in erythrocyte, hemoglobin, and hematocrit levels, was observed throughout the study in rats exposed to 359 mg/kg bw/day and higher, but was not observed in the recovery group. A significant increase in kidney to body weight ratio was observed in rats exposed to 359 mg/kg bw/day and greater from week 79 to 104 (M: 8.1 and 25% and F: 14.4 and 22% increases in 2 highest dose groups, respectively, at week 104). Liver to body weight ratios were significantly increased in both sexes exposed to 359 mg/kg bw/day and higher throughout the study (M: 35 and 61% and F: 26 and 71% increases at 2 highest doses, respectively at week 104). Histological effects observed in kidneys of rats exposed to 359 mg/kg bw/day and higher at weeks 79 and 104 included an increased incidence and severity of renal papilla mineralization in males (59–57/85 at 2 highest doses). An increase in tubule cell pigmentation was also reported by the authors, but the incidence of the lesion appeared equal among control and dose groups (55–59 sex/dose). Urinalysis findings at week 104, which included significant increases in urine output and corresponding

decreases in potassium, calcium, creatinine, and chloride levels in high dose males, suggested compromised ability to concentrate in the renal tubule epithelium. Serum urea levels were significantly increased during the second half of the study in rats exposed to 359 mg/kg bw/day and greater. Increases in urine volume and kidney lesions were observed in the recovery group exposed to 733 mg/kg bw/day and greater with severity approximately equal to that of the 359–442 mg/kg bw/day treatment group. Livers of rats exposed to 359 mg/kg bw/day and higher appeared enlarged and granular at weeks 79 and 104. Histopathological effects in the livers of the high-dose group included diffuse hepatocyte enlargement (37/85 males and 52/85 females), cytoplasmic eosinophilia (43/85 males and 45/85 females), and Kupffer cell/bile canaliculi pigmentation (12/85 males and 17/85 females). These effects were first detected at weeks 2, 13, and 79, respectively. The authors also reported alterations in serum alanine aminotransferase and aspartate aminotransferase activity but the changes did not appear to be consistent or dose related. Non-neoplastic liver changes were found to be reversible in the recovery group. Peroxisomal enzyme activity was significantly increased at week 104 in females exposed to 442 mg/kg bw/day and in both sexes of the high-dose group throughout the study. The recovery group was not tested for peroxisomal enzyme activity. Histopathological changes in testes or female reproductive organs were not observed.

Neoplastic effects included a significant increase in liver adenomas (10/80 vs. 4/80) and carcinomas (11/80 vs. 1/80) in male rats of the high-dose group at week 104. At week 104, renal tubule cell carcinoma was observed in 2 males and mononuclear cell leukemia was found in 45–49% of rats in the top 2 dose groups. Liver neoplasms were not observed in the recovery group but the incidence of renal tubule cell carcinoma in males and mononuclear cell leukemia remained elevated compared to controls. The authors selected a NOAEL of 1,500 ppm (88.3–109 mg/kg bw/day) for this study.

In a 2-year dietary study in B6C3F1/CrlBR mice (*18*) (Table WEB-4), groups of 70 mice/sex/group ate diets that contained 0, 500, 1,500, 4,000, and 8,000 ppm DINP-1 (males: 0, 90.3, 276, 742, 1,560 mg/kg bw/day; females: 0, 112, 336, 910, 1,888 mg/kg bw/day). Body weights and food intake were measured weekly through week 16–17 and monthly thereafter. Standard, hematological, clinical chemistry, and urinalysis parameters were measured every 26 weeks. Peroxisome proliferation was measured in five mice/sex in the control and high-dose group at the midpoint and end of the study. Fifteen mice/sex/group were sacrificed and necropsied at week 79. The remaining mice were sacrificed and necropsied at the end of the 2-year study. Histopathological evaluations were conducted on major organs from mice in all dose groups. Another group of mice was exposed to 8,000 ppm DINP in the diet for 78 weeks and sacrificed at week 105–106 in order to determine if recovery would occur after exposure to DINP ended.

Table WEB-4 (download all WEB-tables separately)

Toxicological and non-neoplastic effects were observed in mice that received the 2 highest doses, 742 mg/kg bw/day and greater. A statistically significant reduction in weight gain occurred throughout the study; this reduction was not accompanied by a decrease in food intake. The effect was shown to be partially reversible because female weight gain in the recovery group was not reduced at week 104; reduced weight gain in males was less pronounced. Clinical signs of toxicity were observed and included abdominal swelling in males exposed to 742 mg/kg bw/day and greater, and hunched posture, decreased activity, and fewer feces in the high-dose males. Survival was significantly reduced in the high dose males (1,560 mg/kg bw/day), with only 63% of males surviving until the end of the study. Survival was not significantly affected in the recovery group. A significant reduction in kidney to body weight ratio was observed in males of the 2 highest dose groups (13 and 25% reduction, respectively), whereas a significant increase in liver to body weight ratio occurred (7 and 24% increase, respectively). Females exposed to the highest dose (1,888 mg/kg bw/day) had a 37% increase in liver to body weight ratio from week 79 to 104. Histological examination revealed an increased incidence and severity of renal nephropathy in female mice of the high-dose group. Urinalysis findings, which included significant increases in urine output and corresponding

decreases in sodium, potassium, and chloride levels in high dose mice from week 52–104, suggested compromised ability to concentrate in the renal tubule epithelium. The effects on renal structure and function proved to be partially reversible as they were less pronounced in mice of the recovery group by the end of the study. Histopathological liver changes were observed in mice of the highest dose group and included diffuse hepatocyte enlargement (56/70 males and 65/70 females) and cytoplasmic eosinophilia (67/70 males and 68/70 females) and pigment (64/70 males and 53/70 females). Other hepatic effects included increased serum alanine aminotransferase and aspartate aminotransferase levels in the high dose males at various time points throughout the study. Non-neoplastic liver changes were found to be reversible in the recovery group. An increase in peroxisomal enzyme activity in mice exposed to 1,560–1,888 mg/kg bw/day indicated that hepatocyte enlargement was due to peroxisomal proliferation. Histopathological changes in testes or female reproductive organs were not observed.

Neoplastic effects included increased incidences of hepatic adenomas and carcinomas combined in females exposed to 336 mg/kg bw/day (10/60 versus 3/70) and adenomas (15/60 and 13/60 versus 10/70) and carcinomas (17/60 and 20/60 versus 10/70) in males exposed to 742 and 1,560 mg/kg bw/day, respectively, and in females exposed to 1,888 mg/kg bw/day (18 adenomas and 18 carcinomas/70 versus 2 adenomas and 1 carinoma/70). The occurrence of hepatic neoplasms was lower in the recovery group compared to the high dose mice exposed for the duration of the study with an incidence of 37–39% versus 50–56%. Based on hepatic neoplasms, the authors selected a NOAEL of 500 ppm (112 mg/kg bw/day) for females and 1,500 ppm (276 mg/kg bw/day) for males.

Hall et al. (*19*) (Table WEB-5) exposed sixteen 25-month-old marmosets (2/sex/group) by gavage for 13 weeks with DINP in 1% methylcellulose and 0.5% Tween at concentrations of 0, 100, 500, or 2,500 mg/kg bw/day. Clofibrate was administered as a positive control at 500 mg/kg bw/day. Analysis was conducted for hematology (weeks 0, 6, and 13), blood chemistry (weeks 0, 4, and 13), estradiol and testosterone levels (week 12), and urine composition (weeks 0, 5, and 12). The main organs were weighed and examined histologically (testes and epididymides were preserved in Bouin's). Peroxisomal proliferation was determined by measuring cyanide-insensitive PCoA oxidase activity.

Table WEB-5 (download all WEB-tables separately)

Clinical signs observed in the marmosets included ungroomed coats and localized reddening of the skin around the anus and legs which was likely caused by excretion of test substance in feces. One male exposed to 2,500 mg/kg bw/day experienced a 13% weight loss and had reduced activity and a hunched posture. Weight loss or decreased weight gain was observed in 2 males and 1 female exposed to 2,500 mg/kg bw/day. Peroxisome proliferation was not evident as indicated by a lack of dose-related increases in PCoA oxidase activity. There were no DINP-treatment related changes in estradiol or testosterone levels, hematology, blood chemistry, organ weights, urine composition, or microscopic findings. The authors identified a NOAEL of 500 mg/kg bw/day.

Administration of the positive control, clofibrate, did result in an approximate 100% increase in PCoA oxidase activity. Other effects in positive control animals included an increase in 11-hydroxylase activity in males, reduced weight gain, anemia, and a slight increase in relative and absolute kidney weight.

See Section 5.1.2 for general toxicity summary.

2.2 Toxicokinetics

Phthalate Associated ADME Toxicokinetics

Absorption

Rodents

Dermal
Dermal absorption of ^{14}C-DINP was studied in male Fischer 344 rats (*20*) in both conditioned (pre-treatment with non-labeled DINP) and non-conditioned skin. Following exposure, the dosed area was occluded. Under all conditions, the amount absorbed after 7 days ranged from 2–4% of the dose. Approximately 93–99% of the administered radioactivity was recovered at the site of application. Radioactivity in feces and gut of the exposed rats suggested some excretion via the biliary route. In *in vitro* studies comparing absorption of DEHP through human and rat skin (*21*), absorption through human skin was slower than through rat skin. Therefore, the dermal absorption rate of DINP is also expected to be slower through human versus rat skin.

Oral
Oral absorption of ^{14}C-DINP was studied (*22*) in conditioned (pre-treatment with non-labeled DINP) and non-conditioned male albino rats. Within 72 hours, 85% of the administered dose was excreted in the feces, most within the first 24 hours. The rest of the dose was excreted in urine (average of 12%) or remained in the tissues (trace amounts). Thus, the oral absorption was approximately 12%. In studies at Midwest Research Institute (*20*), male and female Fischer 344 rats were dosed orally either in a single or in 5 daily doses of 50, 150, or 500 mg/kg. At least 49% of the single low dose was absorbed. Absorption was decreased at the high single dose and at all doses following repeated exposures.

Biotransformation

Most of the ^{14}C collected in the urine of rats following a single oral dose of ^{14}C-DINP was in the form of phthalic acid or side-chain oxidation products of the monoester (MINP) (*20*). The relative amount of phthalic acid in the urine decreased at the high dose. The monoester itself, as well as the diester, was present in only trace amounts. In feces, 8 and 41% of the radioactivity was associated with the diester following administration of a low (50 mg/kg) or a high (500 mg/kg) oral dose of ^{14}C-DINP. This indicates saturation of metabolism at the high dose. The remainder of the fecal radioactivity was associated with the monoester or its side-chain oxidation products. Major metabolites in liver were the monoester and its side-chain oxidation products. The same metabolites were in testes along with phthalic acid. Fat contained the monoester and its oxidation products. Repeated exposures revealed similar metabolites in the tissues. In summary, in the rat, DINP was de-esterified to the monoester, which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. Formation of oxidation products appeared to increase following the high dose or repeated dosing, while the hydrolysis to phthalic acid decreased (*20*)

Distribution

In albino rats receiving 0.5 mL of ^{14}C-DINP after 5 days of dosing with the same amount of unlabeled DINP (*22*), no tissue studied had over 0.001% per gram of the administered dose after 3 days. The liver contained the most radioactivity on a total tissue basis. In male and female Fischer 344 rats receiving single or repeated oral doses of ^{14}C-DINP (*20*), radioactivity also cleared from the tissues rapidly, but analysis of tissues soon (within 1 hour) after the exposure indicated that the highest levels were in liver (4.7% of administered dose), kidneys (0.31%), and blood (1.62 %). Fat and testes contained small amounts of metabolites. No bioaccumulation occurred over 72 hours postdosing.

Excretion

The major routes of excretion for orally administered DINP in rats were urine and feces, with about equal amounts excreted by either route at low doses, but more excreted in feces at high doses (*20*). Repeated dosing caused no accumulation of DINP or its metabolites in blood or tissue, but resulted in increased formation and elimination of the monoester side-chain oxidation products (*20*).

Side-Chain Associated Toxicokinetics

A major metabolite of DINP, the monoester, MINP, is further oxidized in the side chain.

See section 5.1.2 for toxicokinetics summary.

2.3 Genetic Toxicity

DINP was tested in the Ames assay, Chinese hamster ovary (CHO) cells for chromosomal aberrations, the mouse lymphoma forward mutation assay (L5178Y TK =/- cell line), the primary rat hepatocyte unscheduled DNA synthesis assay, and in an *in vitro* transformation assay using clone 1–13 of Balb/c-3T3 A31 mouse cells. Where appropriate, exogenous metabolic activation systems were used. Many of the assays were conducted according to GLP standards (OECD). Based on the results of these studies, DINP is not considered mutagenic in bacterial mutation assays and mammalian gene assays and is not clastogenic in one cytogenetic assay *in vitro* with CHO cells and in one *in vivo* assay with bone marrow cells of Fischer rats. This suggests that DNIP is not genotoxic *in vivo* or *in vitro* (*23*).

Cell transformation studies give various results. The experimental conditions in the assays were not quite identical and the results are not inconsistent. Such positive results are in accord with those of well known peroxisome proliferators (*23*).

Subsequent to the OECD review, DINP tested negative in the L5178 mouse lymphoma mutation assay and the Balb/3T3 cell transformation assay (*24*).

See section 5.1.2 for all summaries addressed under the general and biological and toxicological data section.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

Human data for DINP were not located.

3.2 Experimental Animal Data

Two rat studies evaluating prenatal developmental toxicity of DINP by gavage were reviewed, as were the developmental toxicity aspects of a two-generation study in rats. Prenatal developmental toxicity of isononyl alcohol, a primary metabolite, was also evaluated.

Using Sprague Dawley rats, Waterman et al. (*25*) (Table WEB-7) evaluated DINP 1 (CAS No. 68515-48-0) and diisodecyl phthalate (DIDP) administered by gavage at 0, 100, 500, and 1,000 mg/kg bw/day on gd 6–15, 24 rats/group. For both compounds, maternal toxicity was observed at 1,000 mg/kg bw/day, expressed as reduced weight gain and food consumption. Fetal results were presented in terms of affected litters and fetuses. Skeletal variations were observed at the 500 and 1,000 mg/kg bw/day treatment levels. A dose-related increase in litters with lumbar ribs (25.0, 20.2, 54.2, and 78.3%) was observed that was statistically significant at the high dose. A dose-related increase in the percent of fetuses with rudimentary lumbar ribs, was observed (3.7, 5.4, 18.6, and 34.5%) with statistical significance attained in the mid- and high-dose groups. The percent of fetuses with supernumerary cervical ribs was statistically increased only in the high dose group (1.6, 1.6, 1.0, and 5.7%), but the 2.5-fold increase in litter incidence in the high-dose group was not statistically significant (12.5, 12.0, 8.3, and 30.4%). There was a dose-related increase in the percentage of litters with dilated renal pelves (0, 12.0, 16.7, and 26.1%) that attained statistical significance at the highest dose. The percentage of fetuses with dilated renal pelves was significantly increased at all treatment levels (0, 3.7, 4.0, and 4.5% at 0, 100, 500, and 1,000 mg/kg bw/day, respectively). The interpretation of results by Waterman et al. (*25*) was that a maternal and developmental LOAEL was 1,000 mg/kg bw/day with a NOAEL of 500 mg/kg bw/day, with a conclusion that DINP "is not teratogenic or a selective developmental toxicant." The Expert Panel agreed with the authors selection of a maternal NOAEL; however, the Panel concluded that fetal data indicated that developmental toxicity was present at 500 mg/kg bw/day. The Expert Panel communicated to the study sponsor that there were improved and more current approaches to the analysis of fetal incidence data. The sponsor reanalyzed the fetal incidence data of interest using the GEE approach (*26*). This is a pup-level analysis within a model that uses the generalized estimating equation approach to account for the litter effect, i.e., the correlation between outcomes measured on pups within the same litter. The dose groups were tested pairwise versus controls; this gave similar results to a trend test based on a dose-response model fit with all dose levels up to that of interest included. These reanalysis data, (*27*) presented below, are consistent with the Expert Panel's interpretation of the vertebral data.

Table 5: Mean Percent of Pups in Litter with Effect of Interest (significance level)

		Dose Group (mg/kg bw/day)			
		0	100	500	1,000
DINP	Skeletal Variations	16.4	15.0 (0.91)	28.3* (0.05)	43.4** (0.001)
	Visceral Variations	0.5	3.3 (0.08)	3.7 (0.09)	5.8* (0.04)
	Rudimentary Lumbar Ribs	3.5	4.7 (0.18)	18.1** (0.001)	34.2** (0.001)
	Supernumerary Cervical Ribs	1.6	1.5 (0.81)	1.0 (0.64)	5.5* (0.05)

*≤0.05, **p≤0.01

The GEE methodology could not be used to test the dilated renal pelvis data because of the zero incidence in the control. Two solutions were tried. First, the zero incidence in the control was altered by changing one pup to "affected". Second, an alternative statistical analysis that considers litter effects was used (*28*). The results of these statistical tests show reasonable agreement and are shown below.

Table 6: Mean Percent of Pups in Litter with Dilated Renal Pelves (significance level using two methods)

		Dose Group (mg/kg/day)			
		0	100	500	1000
DINP	Renal Pelves	0.0	3.3	3.7	5.3
	Sig. using added control event		0.06	0.10	0.05*
	sig. using nested analysis		0.18	0.14	0.04*

These results diminish the Expert Panel's initial concern that developmental toxicity effects, based on dilated renal pelves, may extend to lower doses. They now conclude that the 100 mg/kg bw/day dose is a NOAEL.

Hellwig et al. (*29*) (Table WEB-6) evaluated the comparative developmental toxicity of a number of phthalates including three separate DINP materials. The material with CAS No. 68515-48-0 was identified as DINP 1, and two materials with CAS No. 28553-12-0, but from different production lines, were identified as DIDP 2 and DINP 3. See Section 1.1 for description of chemical differences. Each DINP was administered by gavage in olive oil at 0, 40, 200, and 1,000 mg/kg bw/day to 8–10 sperm-positive Wistar females/group on gestational days 6–15. The dams were killed on gd 20 and implantation sites were examined. Fetuses were weighed and examined for external malformations. Half of the fetuses were examined for skeletal malformations and the other half for visceral malformations.

For DINP-1, maternal toxicity at the high dose consisted of reduced food consumption and increased relative liver (~6%; not statistically significant) and kidney (~13%) weights. There were no treatment-related effects on the number of live fetuses/dam or fetal weight. Developmental toxicity was evident at the highest dose by a statistically significant increase in percent fetuses/litter with variations (35.3, 41.5, 29.5, and 58.4% in the 0, 40, 200, and 1,000 mg/kg bw/day groups, respectively). These variations consisted of rudimentary cervical and/or accessory 14th rib(s). There were no maternal or developmental effects at 40 or 200 mg/kg bw/day.

For DINP-2, there was no statistically significant, dose-related evidence of maternal toxicity. However, a non-significant increase in relative liver (~5%) and kidney (~7%) weight did occur. The authors stated that developmental toxicity effects were limited to an increased fetal incidence of accessory 14th lumbar ribs at the high dose.

For DINP-3, maternal toxicity was present at the high dose, expressed as reduced mean body weight gain and reduced food consumption during some portions of the treatment period. Relative liver weights (~11%) were also increased at the high dose and a non-significant increase in relative kidney weights (~9%) was also observed. Developmental toxicity was evidenced by a statistically significant increase in percent fetuses/litter with variations at the highest dose (35.3, 29.6, 39.5, and 60.7% in the 0, 40, 200, and 1,000 mg/kg bw/day groups, respectively). Specific types of developmental toxicity observed in the high-dose group at increased incidences included skeletal retardation (unossified or incompletely ossified sternebrae), and skeletal variations (rudimentary cervical and/or accessory 14th rib[s]). The authors assumed that low incidences of soft tissue variations (hydroureter), visceral malformations affecting the urogenital tract (agenesis of kidneys and ureters), and skeletal malformations affecting the long bones (shortened and bent humerus and femur) observed at the high dose were treatment-related. A maternal and

developmental NOAEL of 200 and LOAEL of 1,000 mg/kg bw/day, respectively, were identified by the Expert Panel and were in concurrence with effect levels identified by authors.

Tables WEB-6 and WEB-7 (download all WEB tables separately)

In a two-generation reproductive toxicity study, postnatal weight gain was examined in pups of dams exposed to DINP in feed at concentrations of 0, 0.2, 0.4, and 0.8 (0, 250, 290, and 500 mg/kg bw/day) during mating, gestation, and lactation (*30*). Complete details of the experiment are included under Section 4. Weight gain for the F_1 pups was reduced by DINP; males of the high-dose group were affected on postnatal day (pnd) 0, pups of the mid- and high-dose groups were affected on pnd 7 and 14 and all dose groups were affected by pnd 21. Weight gain of the F_2 young during lactation was reduced in primarily the mid- and high-dose groups; females of the low-dose group were affected on pnd 7, females of the mid- and high-dose groups were affected on pnds 4, 7, 14, and 21, and males of the mid- and high-dose groups were affected on pnd 7, 14, and 21. Postnatal sexual maturation was not examined.

Hellwig et al. (*31*) evaluated the prenatal development toxicity of two types of isononyl alcohol in Wistar rats. The type 1 alcohol consisted of isomers with a medium degree of branching and 16% isodecanol and the type 2 alcohol consisted of isomers with a low degree of branching. On gd 6–15, 10 rats/group were gavaged with the alcohols in water with 0.005% Cremophor EL at concentrations of 0, 1, 5, and 10 mmol/kg bw/day which the authors stated equated to ~0, 144, 720, and 1440 mg/kg bw/day. A supplementary study was later conducted in 10 rats/group exposed to 0 or type 1 or type 2 alcohol at 7.5 mmol/kg bw/day (~1,080 mg/kg bw/day). In the main and supplementary studies, two groups of 10 control rats each were administered water or vehicle. Fetuses and dams were evaluated on pnd 20.

For the type 1 isononyl alcohol, complete maternal lethality occurred in the 1,440 mg/kg bw/day group and 1 of 10 dams died at the 1,080 mg/kg bw/day dose. Clinical signs/symptoms were reported to have been observed in a dose-related manner in dams that received 720 mg/kg bw/day and the two higher doses. A significant reduction in maternal body weight gain and increased fetal resorptions were observed in the 1,080 mg/kg bw/day group. Numerical increases in fetal resorptions and reductions in fetal body weight occurred in the 720 mg/kg bw/day group. Malformations that primarily affected the heart were significantly increased in fetuses and litters of the 1,080 mg/kg bw/day group. Skeletal variations (cervical ribs) or retardations (reduced ossification of sternebrae) were increased in the 1,080 mg/kg bw/day group (statistically significant) and 720 mg/kg bw/day group.

Maternal mortality was also observed in the 1,440 mg/kg bw/day group treated with type 2 isononyl alcohol with death occurring in 3/10 dams. Maternal signs and symptoms were observed in the three highest doses. Non-significant reductions in body weight gain and marginal increases in resorption rates were observed in dams exposed to 720 mg/kg bw/day and higher. Fetal body weights were significantly reduced in the 1,440 mg/kg bw/day group. The authors reported that malformations were not significantly increased, but, that there were significant increases in fetuses with skeletal variations and retardations (reduced ossification) from the high dose group (1,440 mg/kg bw/day). It is not clear which type of variation was increased. The authors stated the number of fetuses with malformations (primarily affecting the thoracic vertebrae) was elevated in the 1,080 mg/kg bw/day group.

Data available in abstract form (*32*) report that oral exposure of SD rats to DINP at 750 mg/kg bw/day on gd 14 through pnd 3 resulted in reproductive malformations in male offspring (7.7%). The data were not available to the Panel for evaluation, therefore we merely note the existence of the abstract.

See Section 5.1.3 for developmental toxicity summary.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

Data on the reproductive effects of DINP in humans were not located.

4.2 Experimental Animal Toxicity

The reproductive toxicity of DINP-1 (CAS 68515-48-0) was reported by Waterman et al. (*30*) (Table WEB-8). This report describes the results of both a one-generation and a two-generation study. In the two-generation study, SD rats (30/group) were given DINP in the diet at 0.2, 0.4, or 0.8% (w/w) for 10 weeks prior to mating, and through gestation and lactation. Doses were calculated by unconventional methods but were best estimated at 0, 250, 290, and 500 mg/kg bw/day. Body weights and food consumption were measured weekly. After 10 weeks of pre-mating exposure, males and females were paired 1:1 within dose groups, and the females were monitored for vaginal sperm for up to 3 weeks. F_0 males were treated until after the delivery of their last litter and then killed and necropsied; females were killed after weaning their litters. Litters were culled to yield four male and four female pups per litter on pnd 4. At weaning, one male and one female from each litter were selected to grow to adulthood for mating; the remaining animals were examined externally, then killed and discarded without necropsy. F_1 animals were fed the same diet as their parents throughout the rest of the study. As adults, the F_1 rats were mated within dose groups for 3 weeks after confirmation of vaginal sperm. Dams were allowed to litter and raise young until pnd 1, at which time they and their litters were killed, the adults were necropsied, and their organs weighed and preserved. Sperm measures were not made; testes were fixed in Bouin's.

Table WEB-8 (download all WEB tables separately)

Weight gain for the F_0 rats was unaffected by DINP consumption until pnd 14 and 21, when the high-dose dams weighed less than the controls. In the F_0 rats, absolute liver weight was increased in the females of the mid dose group, and in both males and females of the high-dose group. Absolute kidney weight was increased in the upper two dose groups for males and in all exposed female groups. Absolute reproductive organ weights (testes, epididymis, prostate, and seminal vesicles) were unchanged by DINP. At the high dose, absolute left ovary weight was reduced versus control, although the weight of the right ovary was unchanged; the reduced weight of the left ovary appears anomalous. Fertility indices for the F_0 mating were unchanged by DINP; this includes litter size, measures of mating, number of dead offspring, and sex ratio. Weight gain was reduced in both F_1 and F_2 pups and these results are discussed in detail under Section 3. Body weights during the mating of the F_1 generation were variably reduced at the top dose by ~8–10%. When the F_1 animals were mated within dose groups as adults, DINP caused no change in ability to mate or bear young, litter size, pup weight or viability, or sex ratio. At the F_1 adult necropsy, absolute liver weight was increased in the high-dose females and absolute kidney weight was increased in the high-dose males. Absolute reproductive organ weights were unchanged. Livers appeared more eosinophilic in all treated F_0 and F_1 rats; kidneys of the mid- and high-dose males had minimal-to-mild pelvic dilation. Testes were microscopically equivalent to controls.

There is no LOAEL for reproduction, as there were no reproductive toxicities observed. The weight gain inhibition at 0.2% seen by pnd 21 in F1 pups suggests a developmental LOAEL of ~250 mg/kg bw/day. Using benchmark dose methodology (BMD) the authors reported that 250 mg/kg bw/day represented the 95% lower confidence limit for a 5% reduction in body weight.

The NOAEL for reproduction appears to be at least 0.8%, ~500 mg/kg bw/day.

In the one-generation study, groups of 30 male or female animals consumed DINP-1 in the feed at 0, 0.5,1.0, or 1.5% w/w for 10 weeks prior to mating (*30*). Unconventional means of calculating daily doses make it difficult to provide simple dose range estimates. The females were exposed throughout mating, gestation, and lactation until pnd 21. The males were killed immediately after the mating period. At necropsy, the liver, kidneys, and reproductive organs were removed and weighed.

In this one-generation study, body weight gain was reduced at 1 and 1.5% DINP. There were no effects on indices of mating or fertility (litter size), with a reproductive NOAEL of 1,000 mg/kg bw/day. At necropsy, absolute liver and kidney weights were increased in both sexes at all dose levels. Testes absolute weights were increased at the high dose; ovary weights were reduced by ~30% at the highest dose. Offspring viability was reduced in the high-dose group. Offspring body weight gain at pnd 21 was reduced at all dose levels, as in the two-generation study.

Mode of Action

Steroid/Hormone Activity. DINP exhibited no activity in an *in vitro* assay that measured binding of phthalates to estrogen receptors (*33*) and in an assay of estrogen-induced gene expression (*34*). The assays did not include the addition of esterases or lipases to metabolize DINP to MINP. *In vivo* assays demonstrated that DINP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*33*). There were no studies located on anti-androgenic activity but an abstract has reported that gestational DINP exposure demasculinizes male pups (*32*). Thyroid and estrogen serum levels were unaffected in adult marmosets at doses as high as 2,500 mg/kg bw/day for 13 weeks (*19*).

See section 5.1.4 for reproductive toxicity summary.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

DINP, a complex substance of branched, predominantly C-9 isomers (2 CAS RNs–C8-10), is a general-purpose plasticizer for flexible PVC with a broad range of applications. It is widely used in the toy, construction, and general consumer product markets. It has limited use in food packaging. It is not used for medical applications.

Monitoring data are scant. However, the monitoring data for DINP in air, drinking water, and surface and ground waters have usually yielded negative results (i.e., concentrations below detection limits). Because of physicochemical similarities, the pattern of general exposure to DINP is probably very similar to DEHP, and therefore it is anticipated that food contaminated during growth or processing would represent the major human exposure source, followed by indoor air, ambient air, and water. In the few studies of food and infant formula, the levels of DINP have been at or below the detection limit (0.01 mg/kg). Occupational exposures to phthalates are reported to be below 1 mg/m^3 during the production of phthalates and 2 mg/m3 during the manufacture of plasticized PVC (*1*).

Toys represent a unique childhood exposure to DINP since it is the major plasticizer used in children's toys (*5, 6, 9*). DINP content has been measured at 15.1–54.4% dry weight in 31 toys (*5*), and 3.9–44% dry weight in 27/42 toys (*9*). Using pneumatic piston impaction in saliva simulant, DINP migration ranged from

1.0–48.4 μg/11cm^2/hour, but there was no correlation between DINP content and migration rate (*5*). *In vivo* extraction has been studied using adult volunteers as surrogates for children (*5, 12*). In a comparison of extraction rates in 10 adults mouthing toys versus laboratory simulation, ratios varied from 22.9 to 72.6 (mean 39.5) for 5 toys (*5*). RIVM (*12*) tested two different pieces of one toy and a controlled disk using 20 adults and also found higher extraction *in vivo*. Using a 2-day parent observation study of 42 children, ages 3–36 months, mean mouthing times have been generated per age category with ranges from 0 minutes/day in older children to 171.5 minutes/day in the 6–12 month age group (see Section 1). These mouthing times have been used to model DINP exposure by several groups using a variety of assumptions as indicated in Table 7. Dermal exposure may also occur but has not been studied specifically in children.

Table 7: Toy Exposure Estimates for Children Aged 3-12 Months.

	Estimated Intake Level (μg/kg bw/day)		
Agency	Mean	95th Percentile	Maximum
RIVM*	6.53—14.4	20.7—39.7	70.7—204
CPSC	5.7	94.3	-
Health Canada**	44	-	320

* Exposure range for 3-6 month old and 6-12 month old children; range includes results from 3 specimens tested.
** Calculated with mouthing times for teethers and other objects intended for mouthing.

Utility of Data to the CERHR Evaluation. The Expert Panel believes it is reasonable to assume, based on the physicochemical characteristics of DINP and existing, though limited monitoring data, that general population exposure to DINP (excluding children) is expected to be lower than DEHP, which is estimated at 3–30 μg/kg bw/day (*14*). Because extraction of DINP does not correlate with content, because extraction is highly variable across both laboratory procedures and human subjects, and because the number and distribution of children's products containing DINP is unknown, the amounts of DINP presented to a child cannot be well characterized. Furthermore, the estimates of mouthing behavior in the youngest and potentially highest risk group (3–12 months) are based upon 19 children. No discussion of developmental age, physical condition, ethnicity, or other socio-demographic indicators are included in the study of mouthing behavior in children. These estimates of exposures to children through toys are preliminary estimates at best. They do, however, indicate that mouthing of DINP-containing toys may result in significant exposures, potentially in the range of 6–320 μg/kg bw/day in some children.

5.1.2 General Biological and Toxicological Data

General Toxicity. There were no human data identified. Animal data consisted of a subhuman primate study and 4 rodent studies. In a 13-week gavage study, adult marmosets treated with up to 2,500 mg DINP/kg bw/day (CAS number not specified) experienced weight loss or decreased weight gain, but there was no biochemical evidence of peroxisomal proliferation or microscopic changes in organs examined, including testes and epididymides (*19*). A 21-day repeat-dose dietary study in adult rats focused on peroxisome proliferating effects in liver, and a LOAEL of 607 (F) and 639 (M) mg/kg bw/day, was identified; a NOAEL was not established (*15*). Effects included increased liver weight at all dose levels in males and in females, dose-related enzymatic evidence of peroxisomal proliferation, and alterations in hepatic cytoplasmic basophilia and eosinophilia at the high dose. With the exception of severe unilateral atrophy in one male of the mid-dose group, testicular effects were not observed in males dosed with up to 2,195 mg/kg bw/day. Moderate testicular atrophy was observed in one DEHP-positive control that received 1,084 mg/kg bw/day.

There were three chronic (2-year) dietary studies reviewed (*16*) that were of similar design and included toxicopathologic evaluation at several times during the study. Two studies were conducted using 6-week-old F 344 rats (*16, 17*), while the third used 6-week-old B6C3F1 mice (*18*). Lesions in testes or female reproductive organs were not observed in any of the 3 studies, with the highest doses tested being 885 mg/kg bw/day in rats and 1,888 mg/kg bw/day in mice. Non-neoplastic liver lesions and/or changes in liver enzyme activity occurred at doses of 152 mg DINP/kg bw/day and greater in rats, and 1,560 (M) to 1,888 (F) mg/kg bw/day in mice. Biochemical evidence of peroxisomal proliferation was noted throughout the study in both sexes of rats in the Moore (*17*) study that were dosed with 733 (M) and 885 (F) mg/kg bw/day. Female rats receiving 442 mg/kg also had biochemical evidence of peroxisome proliferation when evaluated at the end of the study. Peroxisomal proliferation was noted in high-dose mice (1,560 [M]; 1,888 [F] mg/kg bw/day), but the mid- and low-dose groups were not examined. The Lington et al. (*16*) rat study evaluated peroxisomal proliferation by electron microscopy and saw none in 2 rats per sex per dose group at the end of the study. Non-neoplastic kidney lesions and changes in urinary excretion were seen in rats exposed to 307 mg/kg bw/day and higher, and in mice dosed with 1,560 (M) and 1,888 (F) mg/kg bw/day. Indications of anemia, such as reductions in red blood cell numbers and hemoglobin levels, were seen in rats exposed to 307 mg DINP/kg bw/day and higher. Hepatic neoplasia was observed only in male rats exposed to 733 mg/kg bw/day and in mice exposed to 336 (F) and 742 (M) mg /kg bw/day and greater. Renal neoplasia was only observed in male rats of the highest dose group (733 mg/kg bw/day). The apparent qualitative difference in liver and renal effects (i.e., tumors vs. hepatotoxicity) in the rat studies may reflect differences in the range of doses tested.

Mode of Action. The renal neoplasia in male rats appears to be due to alpha-2-microglobulin nephropathy which is a mechanism not considered relevant to humans (*35*). However, an increased rate of nephropathy, was seen in female mice exposed to 1,888 mg/kg bw/day which would not be consistent with the alpha-2-microglobulin mechanism. The Moore (*17*) study demonstrated liver tumors in rats only in the highest-dose males. Peroxisome proliferation in rats was observed at the highest dose in males and females, and the second highest dose in females but not males. No liver tumors were observed in either sex at the second highest dose level. In addition, no liver tumors were noted in the recovery groups. These results are consistent with a peroxisome proliferation mode of action for hepatic tumor induction. Unfortunately, peroxisome proliferation was assayed in mice only at the highest dose, and liver tumors were observed at lower doses.

Table 8: Summaries of NOAELs and LOAELs and Major Effects in General Toxicity Studies.

Protocol & Study DINP Tested and Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) & Effects	Major Effects at Higher Doses
13-week repeat-dose gavage study in adult marmosets. 16–25 months of age, 1–2 per sex/group. Doses: 0, 100, 500, 2,500. DINP type not specified. (*19*)	500	2,500 ↓Weight gain or weight loss. No peroxisomal proliferation. No microscopic findings in organs.	No higher doses in study.
21-day repeat-dose dietary study in young adult Fischer 344 rats. 6 weeks of age at start of study, 5 rats per sex/group. Doses: M 0, 639, 1,192, 2,195; F 0, 607, 1,193, 2,289. Mixture of different DINP types. (*15*)	None	M: 639, F: 607 ↑ Liver weight, and peroxisomal proliferation. ↑ Kidney weight.	↑ Liver weight, and peroxisomal proliferation. ↑ Kidney weight. ↑Testes weight. No dose-related testicular lesions
2-year repeat-dose dietary study in Fischer 344 rats. 6-week-old at beginning of study, 110 per sex/group. Doses: M: 0, 15, 152, 307; F: 0, 18, 184, 375. DINP-1 (*16*)	M: 15, F: 18	M: 152 F: 184 Hepatic effects. ↑Liver weight. Mononuclear cell leukemia. ↑Kidney weight.	Hepatotoxicity. ↑Liver weight. No treatment related testicular lesions. Mononuclear cell leukemia. Anemia. ↑Kidney weight and excretion changes.
2-year repeat-dose dietary study in Fischer 344 rats. 6 weeks of age at start of study, 70–85 per sex/group. Doses: M 0, 29, 88, 359, 733; F 0, 36, 109, 442, 885. DINP-1 (*17*)	M: 88, F: 109	M: 359, F: 442 Nephrotoxicity. Excretion changes Anemia. ↑ Liver weight, peroxisomal proliferation (F). Mononuclear cell leukemia. ↑Kidney weight.	Hepatic & renal neoplasia at high dose (M). Anemia. Nephrotoxicity. ↑ Liver weight and peroxisomal proliferation. Mononuclear cell leukemia. ↑Kidney weight. No testicular lesions.
2-year repeat-dose dietary study in B6C3F1 mice. 6 weeks of age at beginning of study, 70 per/sex/group. Dose: M 0, 90, 276, 742 or 1,560; F 0, 112, 336, 910, 1,888. DINP-1 (*18*)	M: 276, F: 112	M: 742 F: 336 Liver neoplasia. ↑Liver weight (M) ↓Kidney weight.	Liver neoplasia, hepatocyte staining variations, peroxisomal proliferation, and nephrotoxicty at highest doses. ↑Liver weight ↓Kidney weight. No testicular lesions.

Toxicokinetics. There are no human data. DINP was orally administered to adult male albino rats at doses of 50, 150, or 500 mg/kg bw/day. It is metabolized by pancreatic lipases in the lumen of the gut and rapidly absorbed (49%) as the monoester and rapidly excreted via urine and feces with no accumulation in tissues (*20*). Dermal absorption of DINP is slow (<4% in 7 days) in rats (*20*). Dermal absorption of DINP through human skin is expected to be lower than rat skin based on results of an *in vitro* study conducted with DEHP (*36*). There is evidence for excretion via the biliary route based on radioactivity in feces and GI tract of rats dosed dermally with ^{14}C-DINP. There are no inhalation studies available.

Genetic Toxicity. DINP tested negative in experiments of mutagenicity and clastogenicity including the Ames, Chinese hamster ovary cell (CHO), and rat bone marrow chromosomal aberration, mouse lymphoma mutation, unscheduled DNA synthesis, and Balb/c-3T3 mouse cell transformation assays (*23, 24*)

Utility of Data for the CERHR Evaluation. There are adequate subchronic and chronic data available in rats, mice, and marmosets to assess general toxicity, including liver and kidney effects (*15-18*). No effects have been noted in the male or female reproductive system, although these studies were not designed to fully assess this system.

Toxicokinetic data consist of oral and dermal studies in rodents. The data permit the Panel's conclusion that dermal absorption is slow; oral absorption is rapid for the monoester formed by lipases in the gut. Dose related kinetics of absorption across species is not known. DINP and its metabolites are rapidly excreted via urine and feces with no accumulation in tissues.

5.1.3 Developmental Toxicity

There were no human studies located.

Two published prenatal developmental toxicity studies in rats were available for DINP (*25, 29*). The protocols for the two studies were similar and included dosing of dams by gavage on gd 6–15 with sacrifice and evaluation of fetuses on gd 20–21. The group sizes differed. Developmental toxicity was also noted in both a one-generation and a two-generation toxicity study. The effects on pup body weight are discussed below and summarized in Table 9; the reproductive effects are described in Section 5.1.4.

Hellwig et al. (*29*) performed their studies in Wistar rats (10/group) at doses of 0, 40, 200, and 1,000 mg/kg bw/day. Although sample size (n=10), was small the aggregate of their work can logically be considered to be 3 separate studies of DINP. There was a degree of consistency across all studies. Effects were only observed at the highest dose. Relative kidney and liver weights were slightly increased in dams of the highest dose group (5–13%), but statistical significance was erratic. Fetal viability and body weight were unaffected in all 3 studies. Skeletal variations (rudimentary cervical ribs, accessory 14^{th} ribs) were numerically increased with each DINP with the number of affected fetuses per litter significantly higher than controls in two instances. There was a tendency to see dilated renal pelves at the highest dose; in one study agenesis of kidneys and ureters was assumed by the authors to be DINP-related. Skeletal (shortened and bent humerus and femur) malformations were also observed in the high-dose group of this study. It is clear that organ effects are associated with kidney and the skeletal system. For maternal and developmental effects, a NOAEL of 200 and a LOAEL of 1,000 mg/kg bw/day were identified by the Expert Panel for each DINP and are in concordance with effect levels identified by Hellwig et al. (*29*).

The prenatal toxicity study of Waterman et al. (*25*) was more informative than the Hellwig study from the standpoint of number of rats per test group and completeness of data reported. Waterman et al. (*25*) tested DINP-1 in Sprague-Dawley rats (25/group) at doses of 0, 100, 500, or 1,000 mg/kg bw/day. Maternal toxicity at the highest dose consisted of decreased food intake and weight gain. The authors presented and analyzed effects on offspring as percent affected fetuses and percent affected litters. Waterman et al. (*25*) interpreted their results as indicating a LOAEL for maternal and developmental toxicity at 1,000 mg/kg bw/day and a NOAEL of 500 mg/kg bw/day. The Panel concurred with the maternal NOAEL, but concluded there was developmental toxicity at the 500 mg/kg bw/day dose. As discussed in Section 3.2, the Panel advised the study sponsor that there were more recent and improved methods for the statistical analysis of fetal incidence data. The sponsor performed appropriate reanalyses that the Panel reviewed and found to be consistent with the Panel interpretation of skeletal variations. T he Panel concludes there is a NOAEL in the study at 100 mg/kg bw/day.

The Panel noted that developmental toxicity was observed in the prenatal rat studies by Waterman and Hellwig. In the study by Waterman (n=25), the urinary system was a target of effect as noted by a modest increase in dilated renal pelves at the 1,000 mg/kg dose. While only a mild increase in dilated renal pelves was observed in the three Hellwig et al. studies, in one instance more severe renal effects (hydroureter, agenesis) were seen. In studies by Waterman et al. (*25*) and Hellwig et al. (*29*), the skeletal system was the target for effect as observed by an increased incidence of cervical ribs and accessory 14^{th} (lumbar) ribs. These studies also evaluated the closely related phthalate DIDP where the same target organs were identified. An increase in cervical ribs and lumbar ribs was observed at the common dose of 1,000 mg/kg bw/day in the two studies. While effect on lumbar ribs was more pronounced, the effect on cervical ribs is of greater toxicological concern. Cervical ribs are seen infrequently in controls, but more importantly, their presence may indicate a disruption of gene expression leading to this kind of homeotic anterior/posteriorization. In addition, some scientists express concern that cervical ribs may interfere with normal nerve function and blood flow.

Differences in NOAELs between the Waterman et al. (*25*) and Hellwig et al. (*29*) studies, 100 and 200 mg/kg bw/day respectively, may be due to rat strain, and certainly to dose selection.

The two-generation reproductive study by Waterman et al. (*30*) suggests an adverse effect on weight gain in pups during the perinatal and pre-weaning period of life. Developmental landmarks of reproductive tract development, identified as a sensitive target with other phthalates, were not examined. F_1 mean pup body weight was significantly reduced on pnd 0 in males at 0.8% DINP (~500 mg/kg bw/day during gestation as calculated by authors). On pnd 7 and 14, mean male and female pup body weights were significantly reduced at 0.4% (290 mg/kg bw/day during gestation) and 0.8% and by pnd 21, mean male and female body weights were reduced at all dose levels. In the F_2 generation, mean female pup body weights were significantly reduced at 0.4 and 0.8% on pnd 4, 7, 14, and 21 and at 0.2% (250 mg/kg bw/day during lactation) at pnd 7. Mean male pup body weights were significantly reduced at 0.4 and 0.8% at pnd 7, 14, and 21. The LOAEL for developmental effects was therefore identified as 250 mg/kg bw/day by the Expert Panel.

Studies with 2 isononyl alcohols, differing in degree of branching, demonstrated clinical signs and symptoms in pregnant rats at doses of 720 mg/kg bw/day and higher (*31*). Table and text discrepancies in dose values and reported effects at the higher dose levels were noted. Toxicity was more severe with type 1 isononyl alcohol, the alcohol that had a higher degree of branching. Mortality was seen at the highest dose and in one of the two alcohols at 1,080 mg/kg bw/day. Fetal malformations and variations occurred at the highest dose (1,440 mg/kg bw/day and at 1,080 mg/kg bw/day. Slight effects that may be associated with treatment were observed at 720 mg/kg bw/day. A dose of 144 mg/kg bw/day was without effect for both isonoyl alcohols.

Utility of Data for the CERHR Evaluation. There are adequate data available in rats to determine that prenatal oral exposure to DINP-1 results in developmental toxicity. The results of the Waterman et al. (*25*) and the Hellwig et al. (*29*) studies were remarkably consistent with respect to DINP-1. In both studies, exposure to DINP-1 resulted in increases in lumbar and cervical ribs. In addition, the effective dose levels were similar. Hellwig et al. (*29*) identified a LOAEL of 1,000 mg/kg bw/day and a NOAEL of 200 mg/kg bw/day with a sample size of 10/group. The Panel identified an effect level of 500 mg/kg bw/day from the Waterman et al. (*25*) study (sample size of 25/group) and 100 mg/kg bw/day level represented a NOAEL. In addition, Hellwig et al. (*29*) showed some similarities among the three DINPs in that each resulted in an increase in lumbar and cervical ribs. It is clear that the urinary and skeletal systems are target organs where developmental toxicity is observed. The data from the two-generation dietary study are sufficient to demonstrate an effect on postnatal growth, with a LOAEL of 250 mg/kg bw/day and no NOAEL. The reduced growth is consistent in both studies. Neither prenatal study extended dosing into the late gestation

period which has been shown to be a critical window of development for other phthalates. In addition, the study designs did not allow for assessment of postnatal sexual maturation. The issue of late gestational exposure was addressed in a 2-generation reproductive toxicity study that is reviewed in Section 5.1.4. Confidence in the isononyl alcohol study is limited due to table and text discrepancies in dose values and reported effects at the higher dose levels. The study is adequate to ascribe maternal and developmental toxicity at these higher doses and to assume the lowest dose was without effect.

Table 9: Summary of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies.

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects		Developmental Effects Observed at Higher Dose Levels
		Maternal	Developmental	Developmental
Prenatal gavage study in Wistar rats. 10 per group received 0, 40, 200, or 1,000 mg/kg bw/day on gd 6–15. Dam and pups examined in late gestation. DINP-1, two samples of DINP-2 (*29*)	200 Maternal & Developmental	1,000 ↑Kidney and liver weights.	1,000 ↑Cervical and lumbar ribs –all. ↑Urogenital and skeletal malformation with 1 DIDP.	N/A
Prenatal gavage study in Sprague-Dawley rats. 25 per group received 0, 100, 500, or 1,000 mg/kg bw/day on gd 6–15. Dams & pups examined in late gestation. DINP-1 (*25*)	500 (Maternal) 100 (Developmental).	1,000 ↓Weight gain.	500 ↑ Fetuses with vertebral variations.	↑ Fetuses and litters with visceral variations (mainly dilated renal pelves). ↑ Fetuses and litters with lumbar ribs. ↑ Fetuses with cervical ribs.
Two generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% (~250, 290 or 500 mg/kg bw/day) from 10 weeks prior to mating through gestation and lactation. DINP-1 (*30*)*	None	250 ↑ Mild histologi-cal liver changes in F_0 and F_1. ↑Kidney weight in F_0.	250 ↓ Weight gain on pnd 21in F_1. ↓ Weight gain on pnd 7in F_2 females.	↓ Weight gain on pnd 0 (males), 7, 14, and 21 in F_1. ↓ Weight gain on pnd 4 (female), 7, 14, and 21 in F_2.

*Only maternal and developmental effects were listed in this table. Reproductive and male systemic effects are listed in Table 10.

5.1.4 Reproductive Toxicity

Structural and functional reproductive effects were examined in one- and two-generation feeding studies in rats that included *in utero* exposure during the entire duration of pregnancy (*30*). In the one-generation dose range finding study, rats were administered dietary levels of 0, 0.5, 1.0, or 1.5% DINP and in the two-generation study, rats were administered dietary levels of 0, 0.2, 0.4, or 0.8% DINP. In the two-generation study, reproductive parameters, including mating, fertility, and testicular histology, were unaffected in both generations at the highest dose (0.8%; 500 mg/kg bw/day) and this dose was identified as the reproductive NOAEL. Developmental effects were observed, including decreased pup weight gain (most marked on pnd

21). The effects on pup weight gain are discussed in greater detail under Section 5.1.3, Developmental Toxicity. Histologic effects included mild hepatic eosinophilia in both sexes of parental rats in all dose groups of both generations and dilated renal pelves in F_1 parental males of the mid- and high-dose groups. The results of the study are consistent with the one-generation pilot study that was previously conducted. In the one-generation study, fertility was unaffected in male and female rats exposed to dietary DINP concentrations as high as 1.5% (~1,000 mg/kg bw/day). The findings of these studies indicate that male and female rat fertility and structure of reproductive organs are unaffected by *in utero* exposure to DINP at a maternal dose of ~500 mg/kg bw/day and adult exposure to concentrations as high as ~1,000 mg/kg bw/day.

Mode of Action

Steroid/Hormone Activity. DINP exhibited no activity in an *in vitro* assay that measured binding of phthalates to estrogen receptors (*33*) and in an assay of estrogen-induced gene expression (*34*). The assays did not include the addition of esterases or lipases to metabolize the DINP to MINP. *In vivo* assays demonstrated that DINP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*33*). There were no studies located on anti-androgenic activity, but an abstract has reported that gestational DINP exposure demasculinizes male pups (*32*). Thyroid and estrogen serum levels were unaffected in adult marmosets at doses as high as 2,500 mg/kg bw/day for 13 weeks (*19*).

Table 10: Summary of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies.

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects		Reproductive Effects Observed at Higher Dose Levels
		Repro	Systemic	
Two-generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% (~250–290 or 500 mg/kg bw/day) from 10 weeks prior to mating through gestation and lactation. DINP-1 (*25, 30*)*	500 (Reproductive)	None	250 ↑ Mild liver effects in F_0 and F_1. ↑Kidney weight In F_0 females.	None

*Only effects in parental rats are listed. Developmental effects are listed in Table 9.

Utility of Data for the CERHR Evaluation. The data are sufficient to indicate that DINP exposures are not associated with detectable effects on reproductive function. However, the studies did not assess endpoints of reproductive development shown to be sensitive with other phthalates. The studies did demonstrate consistent effects on the liver (weight and histology) and kidney (weight). Given the constraints of the study design, the data demonstrate no likely reproductive toxicity at 500 mg/kg bw/day in the two-generation study or at 1,000 mg/kg bw/day in the one-generation study.

5.2 Integrated Evaluation

DINP is a complex substance of branched, predominantly C-9 isomers. There are no human data from which to assess the health effects associated with DINP exposure; studies of DINP toxicity are limited to laboratory animals. In the absence of human data, and barring evidence to the contrary, it is assumed that the effects observed in laboratory animals are relevant to humans.

Based upon the physicochemical similarities between DINP and DEHP, and limited DINP monitoring data, general populations exposures to DINP are expected to be lower than those to DEHP which are estimated at 3-30 µg/kg bw/day. It is reasonable to presume that humans would be exposed primarily through the oral route. Oral exposure most likely occurs by the ingestion of food, particularly dairy, meat, fish and poultry, containing DINP from environmental contamination or from food processing. Children may be exposed to higher levels of DINP (up to 10–100 fold higher) than adults for two reasons: 1) disproportionately higher exposure through food due to age related dietary preferences and higher food intake rates per pound body weight and; 2) infants and small children mouth toys that contain DINP that can migrate into saliva and be swallowed. There is no use of DINP in medical devices, therefore intravenous exposure does not occur.

Dermal absorption of DINP is slow in rats. DINP administered orally to rats is metabolized by gut lipases to the monoester, which is rapidly absorbed. DINP and its metabolites are rapidly excreted in urine and feces, with no indication of accumulation in tissues with repeated (5×) daily doses. At low doses, approximately equal amounts of the DINP-derived material is excreted in urine and feces, with the urinary metabolites consisting of the monoester and its oxidation products, while the feces contain those metabolites plus the diester. There are no toxicokinetic studies in humans, but *in vitro* studies comparing the dermal uptake of other phthalates in human and rat skin suggest that dermal uptake of DINP in humans would be negligible.

Oral exposure to DINP has been shown to cause liver and kidney toxicity in adult rats and mice, but not in marmosets. The liver effects are generally consistent with those associated with peroxisome proliferation. Liver tumors have been noted in adult male rats exposed to 733 mg/kg bw/day, in female mice exposed to 336 mg/kg bw/day, and in male mice expand to 742 mg/kg bw/day. Kidney tumors were noted in male rats, but these tumors are associated with a mechanism that is believed not relevant to humans (alpha-2-microglobulin). However, an increased incidence of nephropathy was seen in female mice exposed to 1,888 mg/kg bw/day, which is not consistent with the alpha-2-microglobulin mechanism.

The developmental studies available include examination of effects of prenatal exposure on prenatal development, as well as a limited assessment of postnatal developmental effects in one- and two-generation reproductive studies. The prenatal studies provide consistent results and are sufficient to establish that oral exposure to DINP causes fetal skeletal variations (lumbar and cervical ribs) and in some cases, urinary tract effects (hydroureter). The Panel was confident that 500 mg/kg bw/day was an effect level, and 100 mg/kg bw/day was a NOAEL. For the second developmental toxicity study, the Expert Panel identified a developmental NOAEL of 200 mg/kg bw/day. In addition, the results of the one- and two-generation dietary reproductive toxicity studies demonstrated a consistent reduction in mean pup body weights during lactation at doses as low as 250 mg/kg bw/day and a NOAEL could not be identified. This effect level is similar to that obtained in the more robust prenatal study, although the effects are different and a similar mode of action is not assumed.

There is evidence that isononyl alcohol, a primary metabolite of DINP, is a developmental and maternal toxicant at high (~1,000 mg/kg) oral doses in rats. These doses appear to be greater than the doses of DINP that are associated with developmental toxicity suggesting that effects at lower doses are probably associated with the monoester. The Panel does acknowledge there are no data to permit a judgment as to an interactive effect between the alcohol and monoester metabolite.

Reproductive performance and histological effects on gonads and accessory sex organs were assessed in one- and two-generation dietary studies. Parental doses of up to 0.8% in feed (500 mg/kg bw/day) did not affect fertility or sex organ histology in either the F_0 or F_1 male or female pups. A 13-week gavage study in adult marmosets resulted in no evidence of microscopic testicular changes at doses that did adversely affect body weight gain (2,500 mg/kg bw/day). Chronic 2-year studies in rats and mice gave no gross or histologic evidence of effects on testes or ovaries at doses that did cause liver and kidney effects and other clinical signs of toxicity. Thus, the data are sufficient to conclude that neither the reproductive organs nor fertility is affected by extended oral exposure to DINP. However, the Panel noted that some endpoints, which are sensitive to other phthalates (i.e., preputial separation, nipple retention) were not evaluated in the two-generation study. The Panel is aware that additional data on reproductive tract development are being developed, but as yet only abstracts are available for review. The Panel also notes that the target organs in studies with adult rats, liver and kidney, are also target organs in developmental and multigeneration studies. This increases the Panel's confidence that these effects are real, and that different organ system susceptibilities between adults and young are unlikely.

Summary

DINP is a plasticizer used in flexible polyvinyl chloride (PVC). It is widely used in toy, construction, and general consumer product markets. In adults, exposure to DIDP occurs primarily through food; exposure in children also occurs through food, but additional exposure may occur due to mouthing of toys that contain DINP. While DINP is poorly absorbed through the skin, it is moderately absorbed through the digestive tract. When ingested, a portion of DINP is metabolized to a monoester, MINP, and an isononyl alcohol, and then absorbed into systemic circulation; the remainder is excreted in feces unchanged. In humans, the absorbed monoester is quickly conjugated and excreted. The alcohol is presumed to be metabolized further to the acid and excreted. The Expert Panel believes that general population exposure to DIDP does not exceed exposure estimates of 3–30 μg/kg bw/day, the estimates derived for DEHP.

Most of the toxicological data was collected from studies in rats. There are adequate oral studies to indicate that the major target organ for effects in rats is the liver. Gavage treatment with up to 2,500 mg/kg bw/day produced no evidence of testicular damage in adult marmosets. Dietary exposure to 885 mg/kg did not cause structural changes in reproductive organs in male or female rats or mice, and functional reproductive effects were not observed in male or female rats exposed to 500 mg/kg bw/day.

The data are sufficient to indicate that oral exposure to DINP produced an increase in prenatal developmental variations at doses of 500mg/kg bw/day with a NOAEL of 100 mg/kg bw/day and postnatal effects on body weight gain and liver and kidney weight at a LOAEL of 250 mg/kg bw/day in rats. Structural malformations were not observed. In addition, decreased postnatal weight gain in rat pups was evident from dams exposed to oral doses of 250 mg/kg bw/day during pregnancy and lactation.

Because developmental and functional reproductive effects were only examined in rats, sensitivity in different species is not known. Since the current studies did not assess all endpoints or exposure conditions known to be sensitive to other phthalates, the database used in this review is incomplete. These study results are assumed relevant for evaluating health hazard for humans. These data are sufficient to permit the Panel to conclude that oral exposure to DINP in experimental animal studies indicates that the reproductive system is not a sensitive target for adverse effects. The Expert Panel is confident that DINP may be a developmental toxicant at high doses. The Panel has some uncertainty as to the highest dose without developmental toxicity; it cautiously identified possible effects at low doses. The developmental toxicity LOAELs of 250–500 mg/kg bw/day appear to be 3 or more orders of magnitude greater than the estimated levels of human exposure. Therefore, developmental effects are not likely to occur.

5.3 Critical Data Needs

Critical data needs are discussed under two categories: experimental studies and human exposures.

Experimental studies. Since some relevant endpoints (i.e., nipple retention) were lacking in many of the studies reviewed, uncertainties would be reduced if this additional information were gathered. The Expert Panel recommends a sequential approach for future studies that would focus on obtaining the most critical information first; subsequent studies would be dependent upon the results of the initial study. The Panel further recognized that data gathering should be an iterative process and that recommendations may change as initial tiers of data are gathered. The Expert Panel recommends that the following sequential steps be considered.

1) Conduct a perinatal developmental study, in orally exposed rats, that addresses landmarks of sexual maturation such as nipple retention, anogenital distance, age at testes descent, age at prepuce separation, and structure of the developing reproductive system in pubertal or adult animals exposed through development. Although a two-generation reproductive toxicity study has evaluated some relevant endpoints, the recommended study would provide greater certainty about the lack of such effects with DINP. If DINP does affect these endpoints and the effective dose levels are of possible human health concern, then the Expert Panel recommends that the following study be conducted.

2) Conduct an oral toxicokinetic study, preferably in the rat, then in other species as appropriate (marmoset). There are species differences in the toxicity associated with DINP, and studies with other phthalates suggest that this is partially due to species differences in metabolism, resulting in differences in dose to the target tissues. This is a likely explanation for the lack of toxicity in studies with marmosets. Understanding the toxicokinetics of DINP at different dose levels in various species would therefore reduce uncertainty about the relevance of the data for assessing potential human risk.

3) Conduct a perinatal developmental study with oral exposure in a non-rodent species. There are species differences in the developmental toxicity associated with other phthalates. The developmental effects of DINP have only been examined in the rat. Therefore, there is some uncertainty whether other species would exhibit similar responses and whether the rat is an appropriate model for assessing potential human risk.

Human exposure.

1. It is clear that human exposure to DINP has not been well studied. Direct measurements of DINP content in environmental media are rare; the existing data are mostly estimates.

2. Any investigation of human exposures should begin with the determination of the levels of the agent in environmental media, rather than in biological materials (blood, urine, etc), which is costly and not feasible. Measuring the amount of the agent in media must not be confused with measuring its possible biological effect in humans, or the human response to the agent. These latter studies are not appropriate at present, nor will they be unless a potential exposure source is well documented in a specific group of persons.

3. Patterns of use, expected environmental levels, and vulnerability of exposed population groups should dictate decisions about measuring DINP in environmental media. Due to the DINP use patterns discussed in 1.0, determining DINP exposures for young children and workers engaged in the PVC

production is most important. Out of the two, clearly, the children are more vulnerable and should therefore be the highest priority.

4. Before beginning further assessment of children's toys, manufacturers should be polled to see if DINP will continue to be added to toys in the future. If so, uncertainties such as mouthing times of children need to be better characterized. Such a study is planned by the CPSC.

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